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**"Analysis of Nearest Neighbor Interactions in the Pyrimidine
Triple Helix Motif by Affinity Cleaving"**

by

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Pasadena, CA**

June 1, 1991

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Analysis of Nearest Neighbor Interactions in the Pyrimidine Triple Helix Motif by Affinity Cleaving.

Affinity cleaving studies provide evidence that nearest neighbor interactions affect the relative stabilities of triple helices. Several groups of target duplexes were synthesized with a central base triplet held constant and the adjacent 3' and 5' triplets systematically varied. By incorporating a thymidine residue with the DNA-cleaving moiety EDTA in the third strand, the relative stabilities of Hoogsteen base triplets T·AT and C+GC as well as the newly discovered base triplets G·TA, Z·TA, and D₃·CG were assessed in the context of different sequences. The T·AT triplet was shown to be relatively insensitive to substitutions in either the 3' or 5' directions, while relative stabilities of triple helices containing C+GC triplets decreased as the number of adjacent C+GC triplets increased. Triple helices incorporating a G·TA interaction were most stable when this triplet was flanked by two T·AT triplets, and were most adversely affected when a C+GC triplet was placed in the adjacent 5' direction. In contrast, complexes containing a D₃·TA interaction were destabilized when the adjacent 3' position was occupied with a C+GC triplet. The D₃·CG interaction displayed the same binding preferences. New guidelines for targeting sequences containing pyrimidine base pairs have been developed.



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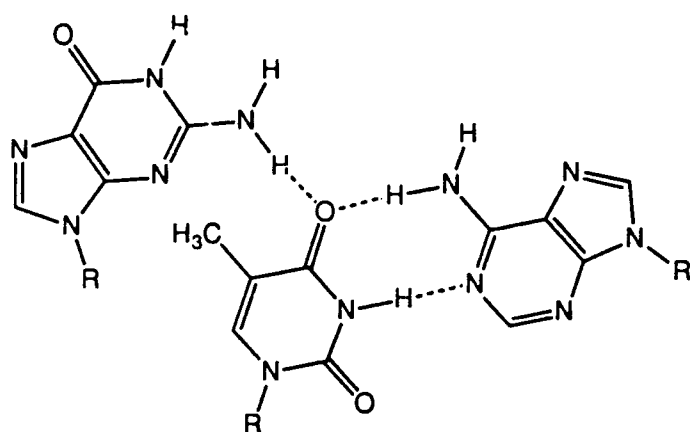
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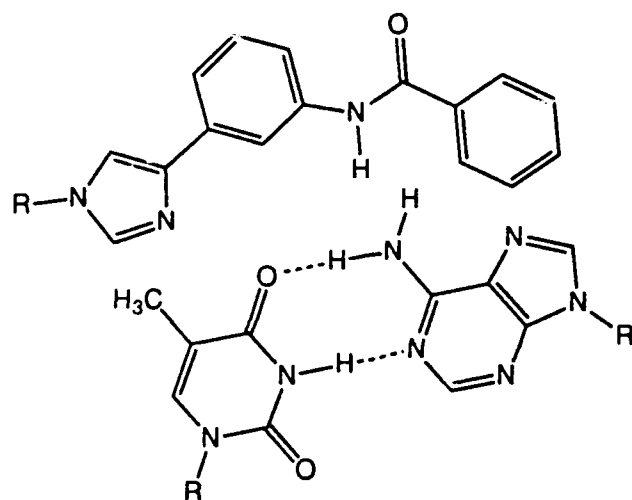
L. L. Kiessling and P. B. Dervan*

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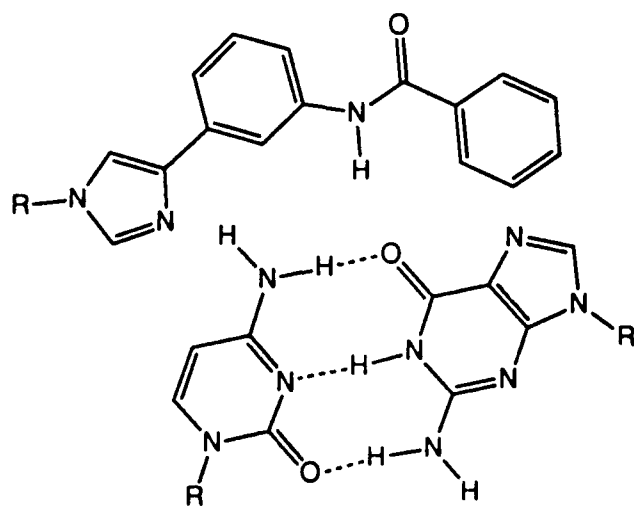
Oligonucleotide-directed sequence specific recognition of double helical DNA is length and sequence composition dependent. In order to dissect the relative importance of nearest neighbor interactions on triplet stabilities, binding by oligonucleotides of different sequence composition has been characterized by affinity cleaving. Several groups of target duplexes were synthesized with a central base triplet held constant and the adjacent 3' and 5' triplets systematically varied. By incorporating a thymidine residue with the DNA-cleaving moiety EDTA in the third strand, the relative stabilities of Hoogsteen base triplets T·AT and C+GC as well as the newly discovered base triplets G·TA, Z·TA, and D₃·CG were assessed in the context of different sequences. The T·AT triplet was shown to be relatively insensitive to substitutions in either the 3' or 5' directions, while relative stabilities of triple helices containing C+GC triplets decreased as the number of adjacent C+GC triplets increased. Triple helices incorporating a G·TA interaction were most stable when this triplet was flanked by two T·AT triplets, and were most adversely affected when a C+GC triplet was placed in the adjacent 5' direction. In contrast, complexes containing a D₃·TA interaction were destabilized when the adjacent 3' position was occupied with a C+GC triplet. The D₃·CG interaction displayed the same binding preferences. New guidelines for targeting sequences containing pyrimidine base pairs have been developed.



G•TA base triplet



D₃•TA base triplet



D₃•CG base triplet

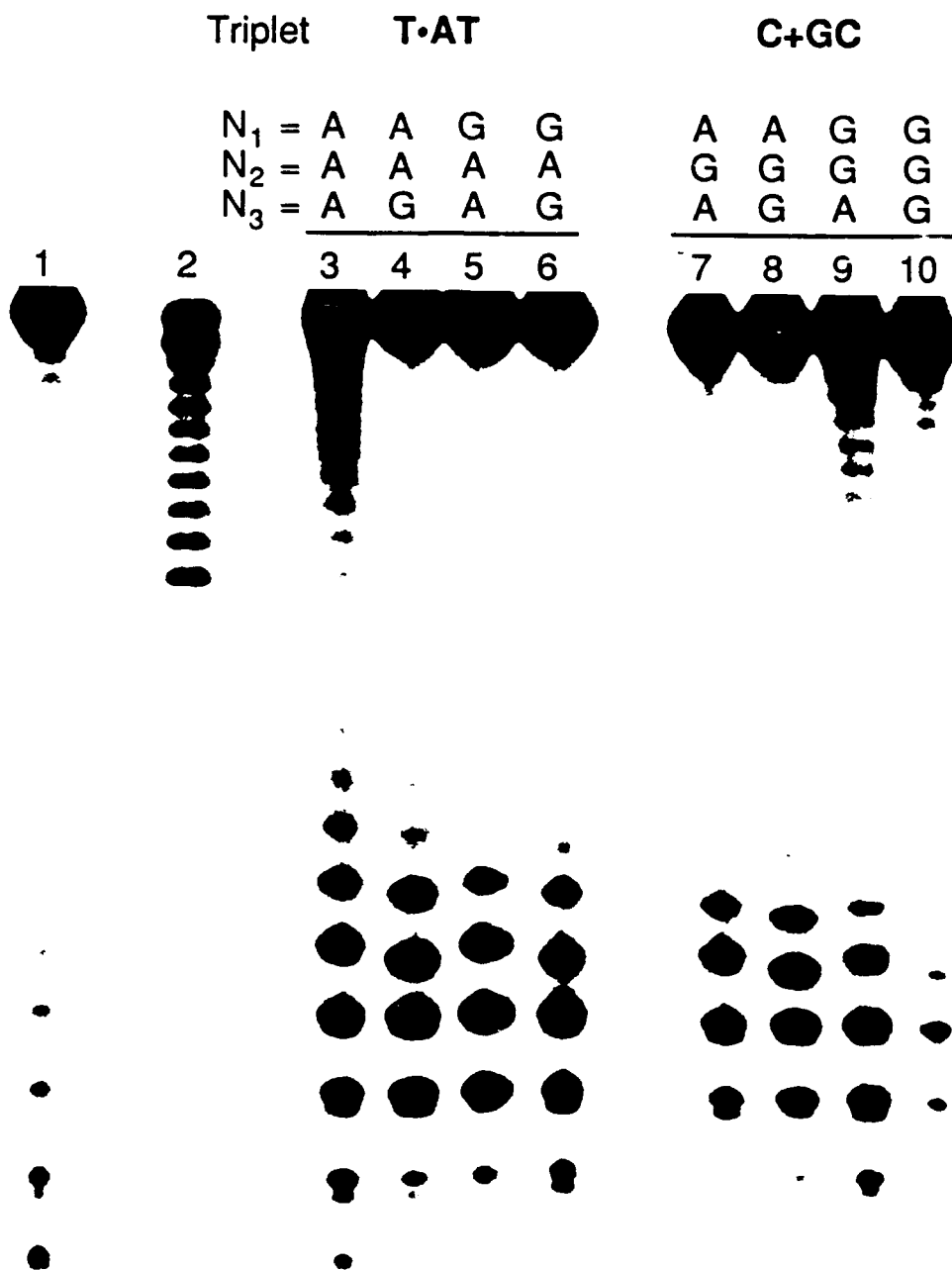
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5'- T T T T T [*] T T T C T T T T T T T T -3'	5
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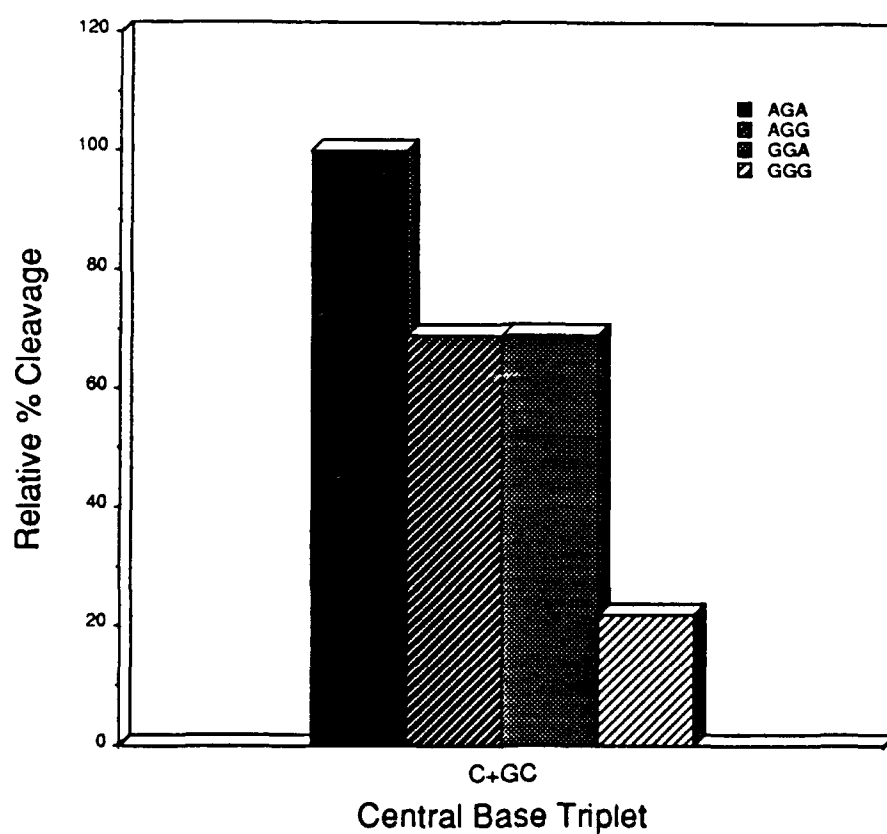
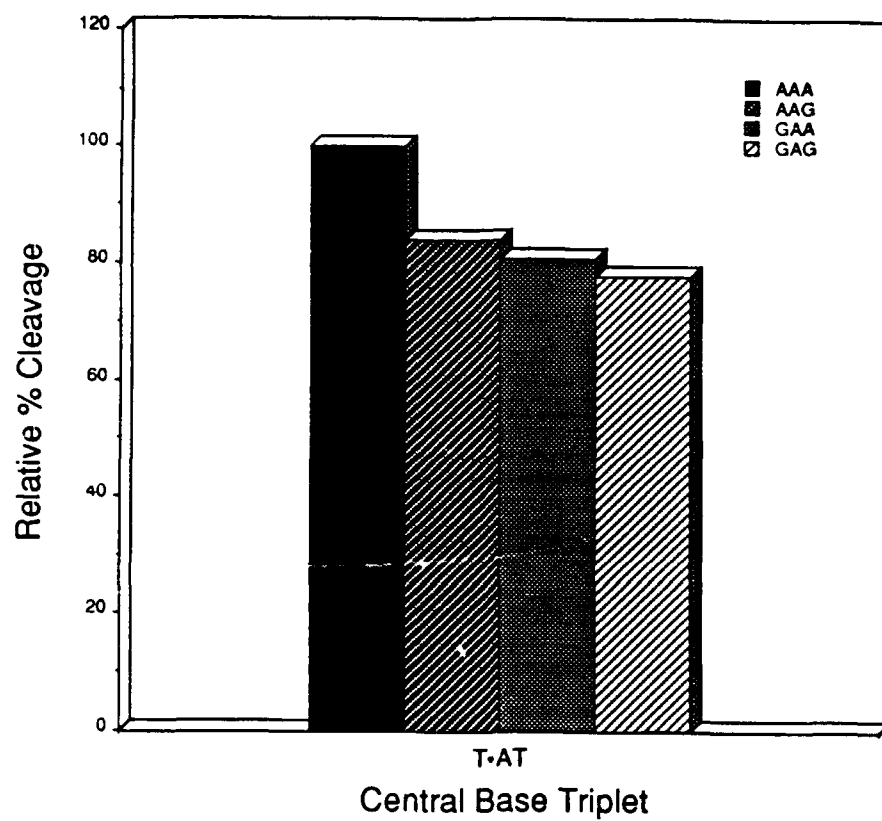
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3'- G G G G G G G G G G T T T T T N₆ N₅ N₄ T T T T T A A A A A -5'



N ₁ N ₂ N ₃ = AAA 9a	N ₄ N ₅ N ₆ = TTT 9b
N ₁ N ₂ N ₃ = AAG 10a	N ₄ N ₅ N ₆ = CTT 10b
N ₁ N ₂ N ₃ = GAA 11a	N ₄ N ₅ N ₆ = TTC 11b
N ₁ N ₂ N ₃ = GAG 12a	N ₄ N ₅ N ₆ = CTC 12b
N ₁ N ₂ N ₃ = AGA 13a	N ₄ N ₅ N ₆ = TCT 13b
N ₁ N ₂ N ₃ = AGG 14a	N ₁ N ₅ N ₆ = CCT 14b
N ₁ N ₂ N ₃ = GGA 15a	N ₄ N ₅ N ₆ = TCC 15b
N ₁ N ₂ N ₃ = GGG 16a	N ₄ N ₅ N ₆ = CCC 16b





5'- T T T T ^{*} T T T T T T T T T T -3' 17
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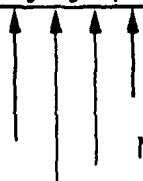


N₁N₂N₃ = ATA 25a
 N₁N₂N₃ = ATG 26a
 N₁N₂N₃ = GTA 27a
 N₁N₂N₃ = GTG 28a

N₄N₅N₆ = TAT 25b
 N₄N₅N₆ = CAT 26b
 N₄N₅N₆ = TAC 27b
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 5'- T T T T ^{*} T T C D₃ T T T T T T T T T -3' 31
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N₁N₂N₃ = ATA 25a
 N₁N₂N₃ = ATG 26a
 N₁N₂N₃ = GTA 27a
 N₁N₂N₃ = GTG 28a
 N₁N₂N₃ = ACA 33a
 N₁N₂N₃ = ACG 34a
 N₁N₂N₃ = GCA 35a
 N₁N₂N₃ = GCG 36a

N₄N₅N₆ = TAT 25b
 N₄N₅N₆ = CAT 26b
 N₄N₅N₆ = TAC 27b
 N₄N₅N₆ = CAC 28b
 N₄N₅N₆ = TGT 33b
 N₄N₅N₆ = CGT 34b
 N₄N₅N₆ = TGC 35b
 N₄N₅N₆ = CGC 36b

Triplet

I•TA

G•TA

D₃•TA

D₃•CG

N₁= A A G G
N₂= T T T T
N₃= A G A G

A A G G
T T T T
A G A G

A A G G
T T T T
A G A G

A A G G
C C T T
A G A G

1

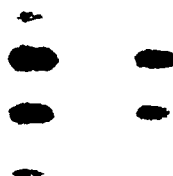
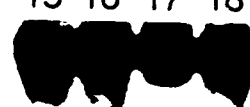
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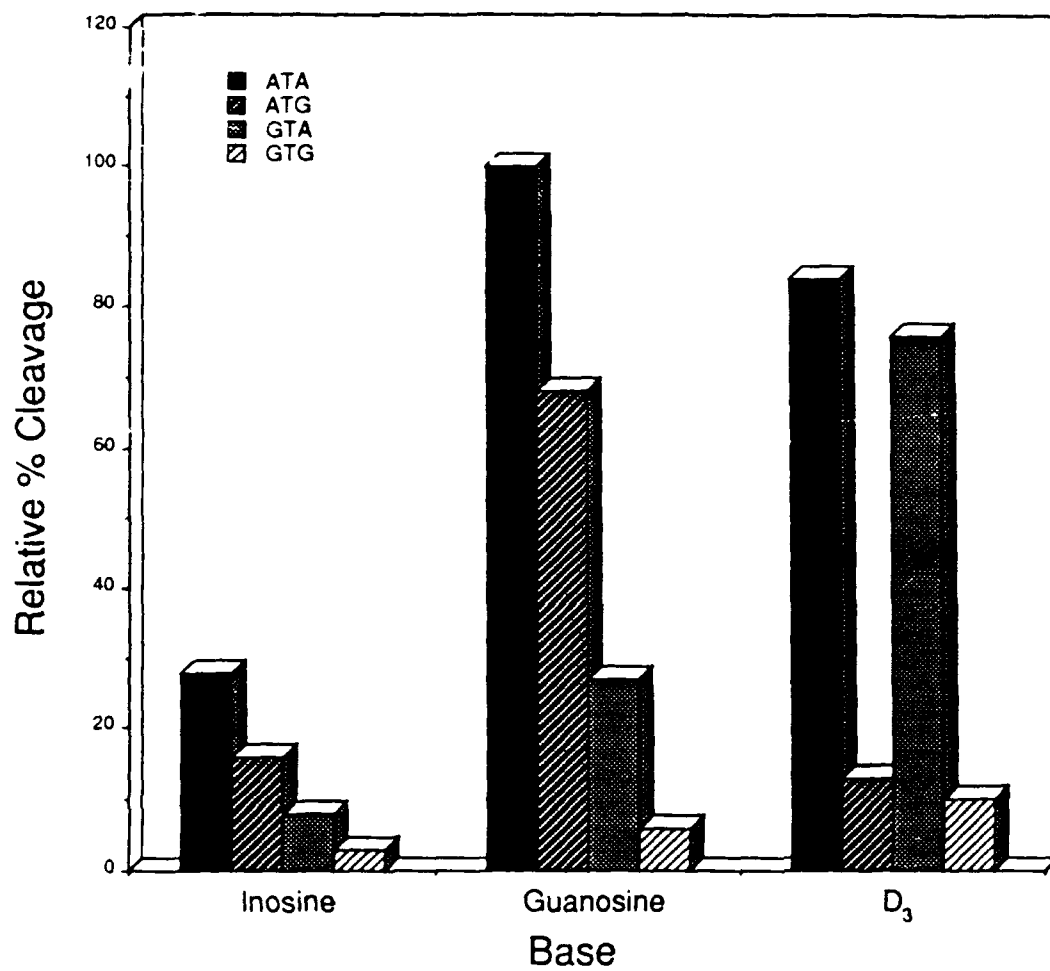
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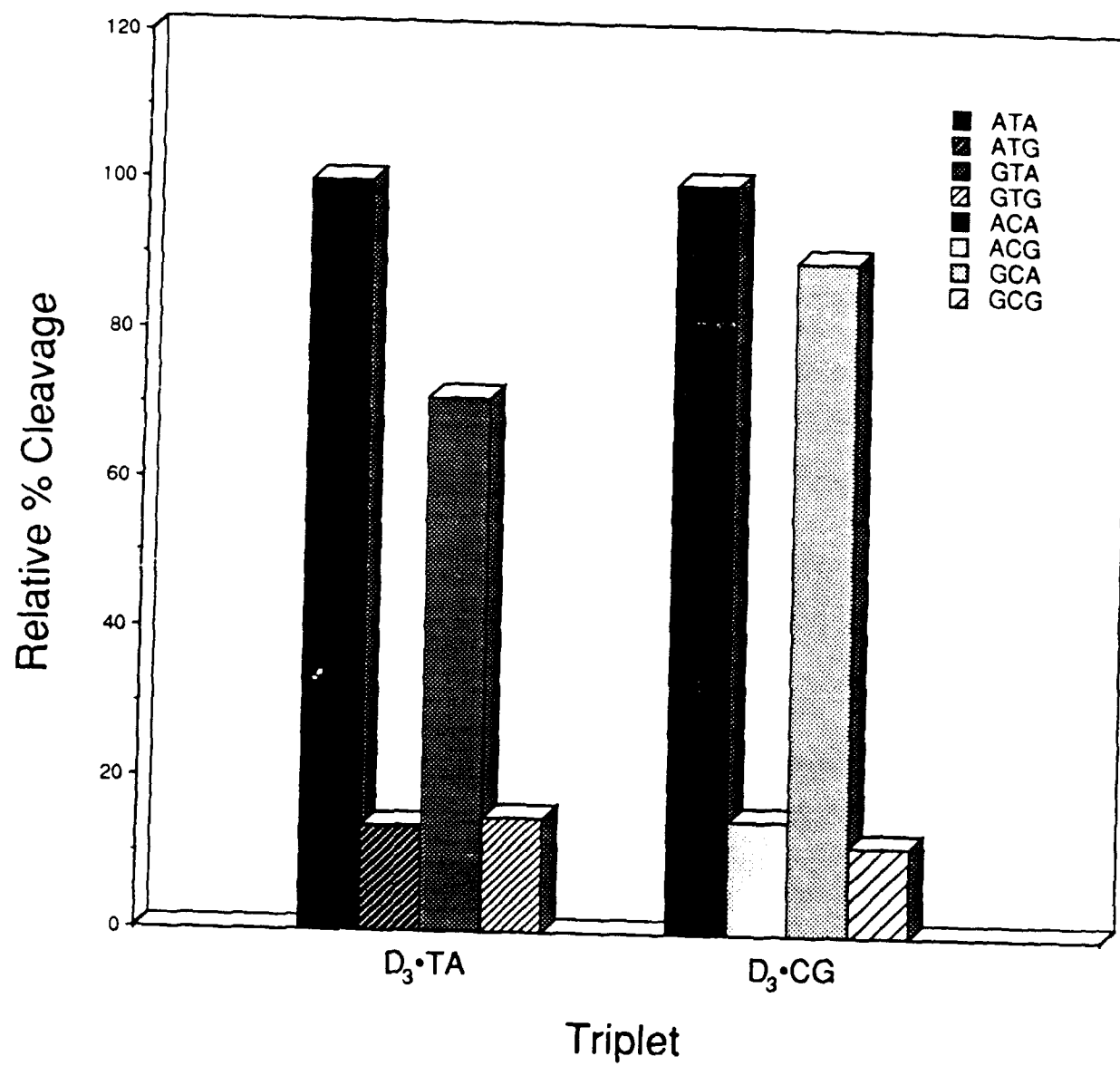
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11 12 13 14

15 16 17 18







Target Sequence	G	D ₃
5'- ATA -3'	+++	+++
ATG	++	+
GTA	+	+++
GTG	-	-
5'- ACA -3'	-	+++
ACG	-	+
GCA	-	+++
GCG	-	-